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CD4 T cells in hepatitis B virus: “you don’t have to be cytotoxic to work here and help”.  
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**EDITORIAL TITLE: CD4 T cells in hepatitis B virus: “you don’t have to be cytotoxic to work here and help”.**

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Understanding the immunopathogenesis of chronic hepatitis B (CHB) in full will be key to delivering the hepatitis B virus (HBV)-cure program, allowing us to induce a range of host-leukocyte responses that act in concert to achieve complete viral elimination. While previous research has understandably focused on restoring the function of exhausted virus-specific CD8 T cells, there has been a relative lack of appreciation for the role played by the CD4 T cell pool and virus-specific CD4<sup>+</sup> clones in controlling HBV infection [1]. It is well established that CD4 T cells exert a multitude of critical functions in host immunity, including promotion of B cell antibody production, driving granulocyte recruitment to sites of infection and supporting activation of antigen presenting cell (APC) populations via CD40:CD40L interactions [2]. Of particular relevance in the context of CHB is the ability of naïve CD4 T cells to differentiate into functionally distinct subsets under the control of specific transcription factors and polarising cytokines. Major subtypes include T-helper (Th)1 cells which produce IFN $\gamma$ /IL-12, Th2 mainly producing IL-4, Th17 producing IL-17/IL-21 (dependent upon ROR $\gamma$ T), and the T-regulatory (T-reg) population which depends on FoxP3. Despite the recent focus on CD8 T cell function in HBV research, data from

the 1980's has previously indicated that CD4 T cells are the dominant supporters of CD8 T cell-mediated HBV responses and play essential roles in driving CD8 T cell effector function and memory formation [3]. Indeed, lack of CD4 T cells was even considered to be a major cause of exhaustion among virus-specific CD8 T cells, which are dependent upon a continued supply of multiple cytokines to maintain their effector functions [4]. In line with this concept, other investigators have also observed that loss of CD4 T cells during acute infection can generate a defective memory CD8 T cell compartment, including the loss of IL-2 production, thus mounting only weak responses upon secondary pathogen exposure [5]. While chronic viral infections clearly have potential to induce exhaustion in CD4 T cell populations similarly to their CD8 T cell counterparts, this area is notably understudied at present. A recent report indicated that during chronic infection, CD4 T cells display increased expression of mRNAs encoding transcription factors implicated in the development of different Th cell subsets. These data indicate that exhausted CD4 T cells do not merely diminish in their ability to produce cytokines - they also exhibit an altered functional profile as a consequence of change in differentiation patterns [6]. In particular, CD4 T cells have been shown to act as potent producers of IL-21 which likely sustains CD8 T cell responses to chronic infections, hence differentiation towards this functional profile may be an important determinant of patient outcome. In the absence of IL-21 derived from CD4 T cells, virus-specific CD8 T cells appear to lose their ability to produce IL-2, TNF $\alpha$  and IFN $\gamma$  and display other hallmarks of an exhausted state (including high levels of CD43 and PD-1) [7]. Consistent with this finding, a recent study in CHB patients showed that combined OX40 stimulation and PD-L1 blockade significantly augmented IFN $\gamma$  and IL-21 producing HBV-specific CD4 T cells *in vitro*, suggesting that such immunotherapeutic approaches could potentially also improve virus-specific CD4 T cells responses *in vivo* [8].

Seroclearance of chronic viral infection is thought to be mediated primarily by IFN $\gamma$  release, but the CD4 T cell pool may simultaneously exacerbate immunopathology via production of TNF $\alpha$  which potentially worsens hepatic injury (as previously described in a mouse model of concanavalin-A induced hepatitis [9]). Indeed, a recent study identified that IL-17 producing CD4 T cells (Th17) enriched in the blood and liver of CHB patients are closely associated with hepatic flares, during which they display marked expression of the pro-inflammatory cytokines IL-1 $\beta$ , IL-6, TNF $\alpha$  [10]. Multiple other studies also suggest that the CD4 T cell compartment may represent an important 'inflammatory' component of CHB pathology, including an increased expression of NKG2D ligands which blunt HBV-specific CD4 T cell responses with their interaction of the NKG2D receptor on NK cells [11]. Whether operating in a regulatory or pro-inflammatory role, it is clear that CD4 T cells have a major part to play in mediating viral clearance and control, but until recently studies in CHB have been lacking.

In this edition of *Journal of Hepatology*, Wang *et al.*, perform a thorough immunological analysis of the HBV-specific CD4 T cell response in patients in various disease phases of CHB. They report that distinct subsets of HBV-specific CD4 T cells producing TNF $\alpha$  or IFN $\gamma$  are associated with liver damage or viral clearance, respectively. The authors utilised a panel of HLA-restricted epitopes against CD4 T cells and undertook analysis with these HBV peptides against the core (HBc) and surface (HBs) (envelope) proteins [12]. A previous study has described

that increasing CD4 T cell responses to HBc and HBeAg occur during seroconversion to anti-HBe and anti-HBs production in acute HBV infection, implying that T cells directed against these proteins are essential for viral elimination. In contrast, patients with CHB failed to mount an efficient T cell response to HBc/HBs, thereby contributing to persistence of infection [13]. Wang *et al.*, report a significant overall expansion of TNF $\alpha$ -producing, compared to IFN $\gamma$ -expressing, HBc- and HBs-specific CD4 T cells in their patient cohort. Accounting for patient clinical parameters, the authors report a dominance of TNF $\alpha$ -producing HBV-specific CD4 T cells in HBeAg+ patients with high viral load, whereas IFN $\gamma$ -expressing HBV-specific CD4 T cells instead dominated in patients who approached viral clearance (HBeAg seroconversion and HBsAg loss). Importantly HBsAg level negatively correlated with the dominance of IFN $\gamma$ + HBV-surface specific CD4 T cells. Thus viral clearance is accompanied by an elevation in both the frequency and dominance of antigen-specific CD4 T cells producing IFN $\gamma$ , which has also been observed for other viral infections such as CMV [14] and bacterial infections [15].

It is now widely accepted that induction of hepatic flares in CHB may in fact aid seroclearance (HBsAg loss), such that discontinuation of nucleos(t)ide analogue (NA) therapy has now been described as a potential therapeutic strategy in a number of different studies [16]. In order to circumvent potential for excessive hepatic flares and possible liver failure, studies of treatment discontinuation are undertaken in a controlled manner and thus large flares are carefully avoided. Coinciding with this, virus-specific CD4 T cell responses in patients stopping NA therapy with a mild-moderate rise in transaminases demonstrate only limited virus-specific CD4 T cell responses [17, 18]. It is notable therefore that Wang *et al.*, studied patients presenting with severe hepatic flares prior to commencing NA therapy. Consequently, they observed potent TNF $\alpha$ + (core>surface) virus-specific CD4 T cell responses in patients with aggressive liver damage, followed by significant decreases in these responses only after NA introduction. In contrast, IFN $\gamma$ + virus-specific CD4 T cell responses in these patients were characteristic of individuals capable of clearing HBsAg [12]. These findings are consistent with previous reports of high circulating serum TNF $\alpha$  in patients with active hepatitis B [19], indicating that this cytokine may contribute to liver damage and immunopathology (presumably with a view to gaining viral control and allowing for subsequent induction of virus-specific T cells expressing IFN $\gamma$ ). Of note, TNF $\alpha$ /IFN $\gamma$  double producers and IFN $\gamma$ + single producers were routinely detected in patients undergoing HBeAg and HBsAg seroconversion, suggesting that antigen specific-CD4 T cell responses transition from TNF $\alpha$  to IFN $\gamma$  expression in individuals undergoing viral clearance (*Figure 1*). Both populations were observed to express the lineage-specific transcription factors, T-bet and ROR $\gamma$ T at comparable levels, suggesting that Th1 and Th17 pathways of differentiation may be important in this context (although IL-21 rather than IL-17 appeared critical in this case). Further work will now be required to elucidate this process in full and identify the transcription factor profile associated with mono-production of TNF $\alpha$ .

The importance of T cell differentiation in shaping the cytokine responses that switch patients from an inflammatory profile (TNF $\alpha$  dominated) to achieving viral clearance (IFN $\gamma$ ) has been underappreciated in the context of CHB pathology and therapy. The authors have shed new light on the profile of CD4 T cells in CHB and

're'-opened important avenues for the future study of these crucial cells. This work should encourage other researchers in the field to now consider the influence of CD4 T cell differentiation pathways and downstream interactions with CD8 T, B and innate leukocyte populations on anti-viral responses. Such analyses should not only be undertaken in the blood but also within the intrahepatic compartment, both in treatment-naïve subjects and those undergoing therapy via longitudinal sampling [20]. Ultimately, therapeutic approaches that can increase T-bet expression and enhance IL-21 synthesis in patient CD4 T cells may succeed in boosting virus-specific CD8 T cell responses and augmenting strategies for HBV cure.

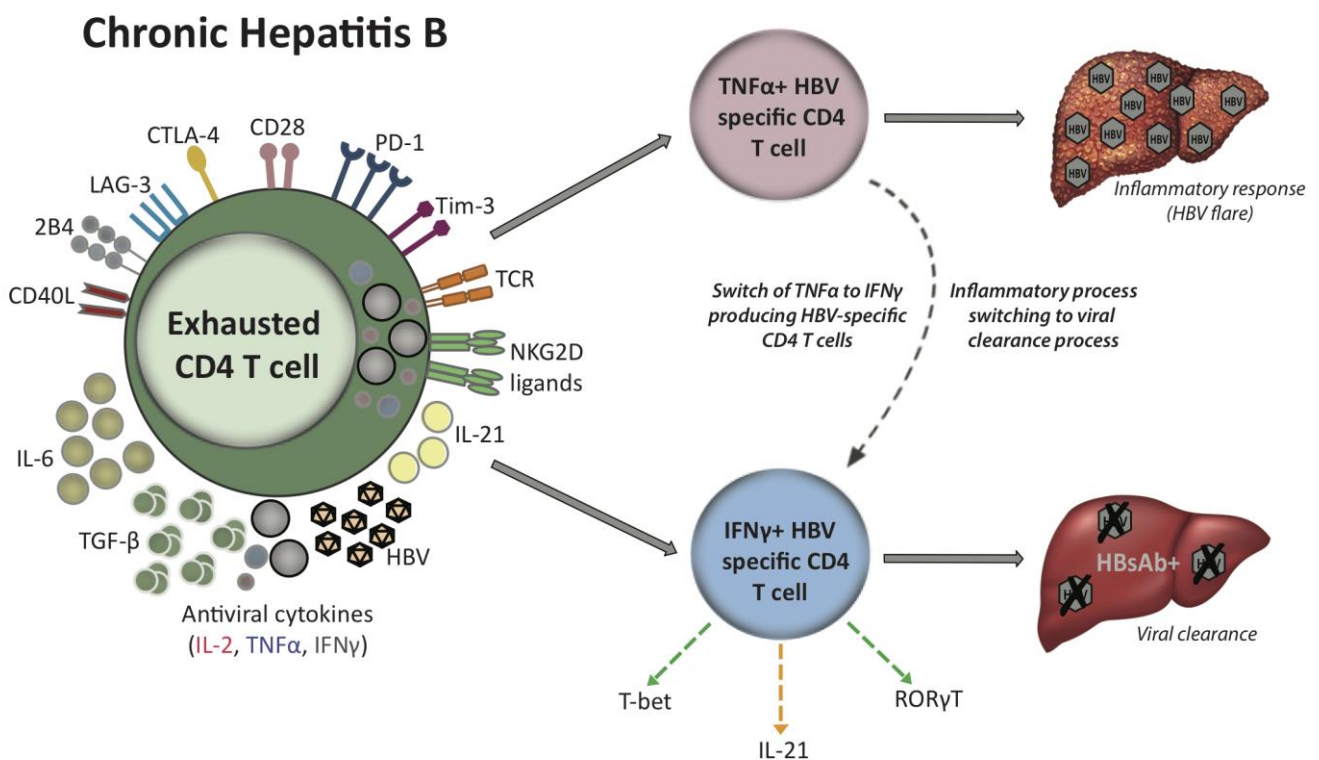


Figure 1: Diagram depicting the phenotype of an exhausted CD4 T cell in chronic hepatitis B infection. The expression of TNFα/IFNγ producing HBV-specific CD4 T cells during HBV infection is represented and how this leads to the generation of an inflammatory response or that of viral clearance respectively. A switching of cytokine producing from TNFα to IFNγ producing virus specific CD4 T cells may progress towards viral clearance, with the expression of other cytokines and transcriptions factors governing the differentiation process.

## References:

1. Raziorrouh B, Heeg M, Kurktschiev P, Schraut W, Zachoval R, Wendtner C, Wachtler M, Spannagl M, Denk G, Ulsenheimer A *et al*: **Inhibitory phenotype of HBV-specific CD4+ T-cells is characterized by high PD-1 expression but absent coregulation of multiple inhibitory molecules.** *PLoS One* 2014, **9**(8):e105703.
2. Zhu J, Paul WE: **CD4 T cells: fates, functions, and faults.** *Blood* 2008, **112**(5):1557-1569.
3. Fang D, Zhu J: **Dynamic balance between master transcription factors determines the fates and functions of CD4 T cell and innate lymphoid cell subsets.** *J Exp Med* 2017, **214**(7):1861-1876.
4. Saeidi A, Zandi K, Cheok YY, Saeidi H, Wong WF, Lee CYQ, Cheong HC, Yong YK, Larsson M, Shankar EM: **T-Cell Exhaustion in Chronic Infections: Reversing the State of Exhaustion and Reinvigorating Optimal Protective Immune Responses.** *Front Immunol* 2018, **9**:2569.
5. Bevan MJ: **Helping the CD8(+) T-cell response.** *Nat Rev Immunol* 2004, **4**(8):595-602.
6. Crawford A, Angelosanto JM, Kao C, Doering TA, Odorizzi PM, Barnett BE, Wherry EJ: **Molecular and transcriptional basis of CD4(+) T cell dysfunction during chronic infection.** *Immunity* 2014, **40**(2):289-302.
7. Elsaesser H, Sauer K, Brooks DG: **IL-21 is required to control chronic viral infection.** *Science* 2009, **324**(5934):1569-1572.
8. Jacobi FJ, Wild K, Smits M, Zoldan K, Csernalabics B, Flecken T, Lang J, Ehrenmann P, Emmerich F, Hofmann M *et al*: **OX40 stimulation and PD-L1 blockade synergistically augment HBV-specific CD4 T cells in patients with HBeAg-negative infection.** *J Hepatol* 2019, **70**(6):1103-1113.
9. Kusters S, Tiegs G, Alexopoulou L, Pasparakis M, Douni E, Kunstle G, Bluethmann H, Wendel A, Pfizenmaier K, Kollias G *et al*: **In vivo evidence for a functional role of both tumor necrosis factor (TNF) receptors and transmembrane TNF in experimental hepatitis.** *Eur J Immunol* 1997, **27**(11):2870-2875.
10. Zhang JY, Zhang Z, Lin F, Zou ZS, Xu RN, Jin L, Fu JL, Shi F, Shi M, Wang HF *et al*: **Interleukin-17-producing CD4(+) T cells increase with severity of liver damage in patients with chronic hepatitis B.** *Hepatology* 2010, **51**(1):81-91.
11. Huang WC, Easom NJ, Tang XZ, Gill US, Singh H, Robertson F, Chang C, Trowsdale J, Davidson BR, Rosenberg WM *et al*: **T Cells Infiltrating Diseased Liver Express Ligands for the NKG2D Stress Surveillance System.** *J Immunol* 2017, **198**(3):1172-1182.
12. Wang H, Luo H, Wan X, Fu X, Mao Q, Xiang X, Zhou Y, He W, Zhang J, Guo Y *et al*: **TNF-alpha/IFN-gamma profile of HBV-specific CD4 T cells is associated with liver damage and viral clearance in chronic HBV infection.** *J Hepatol* 2019.
13. Jung MC, Diepolder HM, Spengler U, Wierenga EA, Zachoval R, Hoffmann RM, Eichenlaub D, Frosner G, Will H, Pape GR: **Activation of a heterogeneous hepatitis B (HB) core and e antigen-specific CD4+ T-cell population during seroconversion to anti-HBe and anti-HBs in hepatitis B virus infection.** *J Virol* 1995, **69**(6):3358-3368.
14. Kannanganat S, Ibegbu C, Chennareddi L, Robinson HL, Amara RR: **Multiple-cytokine-producing antiviral CD4 T cells are functionally superior to single-cytokine-producing cells.** *J Virol* 2007, **81**(16):8468-8476.
15. Green AM, Difazio R, Flynn JL: **IFN-gamma from CD4 T cells is essential for host survival and enhances CD8 T cell function during Mycobacterium tuberculosis infection.** *J Immunol* 2013, **190**(1):270-277.
16. Papatheodoridis G, Vlachogiannakos I, Cholongitas E, Wursthorn K, Thomadakis C, Touloumi G, Petersen J: **Discontinuation of oral antivirals in chronic hepatitis B: A systematic review.** *Hepatology* 2016, **63**(5):1481-1492.
17. Rivino L, Le Bert N, Gill US, Kunasegaran K, Cheng Y, Tan DZ, Becht E, Hansi NK, Foster GR, Su TH *et al*: **Hepatitis B virus-specific T cells associate with viral control upon nucleos(t)ide-analogue therapy discontinuation.** *J Clin Invest* 2018, **128**(2):668-681.
18. Rinker F, Zimmer CL, Honer Zu Siederdisen C, Manns MP, Kraft ARM, Wedemeyer H, Bjorkstrom NK, Cornberg M: **Hepatitis B virus-specific T cell responses after stopping nucleos(t)ide analogue therapy in HBeAg-negative chronic hepatitis B.** *J Hepatol* 2018, **69**(3):584-593.
19. Fang JW, Shen WW, Meager A, Lau JY: **Activation of the tumor necrosis factor-alpha system in the liver in chronic hepatitis B virus infection.** *Am J Gastroenterol* 1996, **91**(4):748-753.
20. Gill US, Pallett LJ, Thomas N, Burton AR, Patel AA, Yona S, Kennedy PTF, Maini MK: **Fine needle aspirates comprehensively sample intrahepatic immunity.** *Gut* 2019, **68**(8):1493-1503.